Characteristics of A Red-Eye Mutant of the Tarnished Plant Bug (Heteroptera: Miridae)

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ABSTRACT   Normal eye color of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is dark purplish-black. A strain homozygous for a new mutation, red eye color, was established and reared in the laboratory for >5 yr. The mutant phenotype is controlled by a single autosomal recessive allele. The gene symbol assigned to this new mutant is *r.* Laboratory tests showed that egg production and egg viability in the red-eye mutant females were not different from that of normal-eye females. Expression of the red-eye mutation was not found in extensive samples of wild populations in the Mississippi River Delta, and was rare in populations near Crossett, AR, where the original mutant was found. The mutant strain could be of use as a genetic marker in studying reproductive behavior and population dynamics of this important pest species.

KEY WORDS   *Lygus lineolaris*, tarnished plant bug, mutants, eye color

THE TARNISHED PLANT BUG, *Lygus lineolaris* (Palisot de Beauvois), is found in all agricultural regions of the United States and Canada (Kelton 1975). It has been reported to feed on 328 different plant species, and is an economically important pest of many crops (Young 1986). Tarnished plant bugs have been reared in the laboratory as part of studies on their biology since the early 1900s (Crosby and Leonard 1914), and in numerous other studies since then (Ridgway and Gyrisco 1960, Vanderzant 1967, Stevensen and Roberts 1973, Wilson 1973, Khattat and Stewart 1977, Snodgrass and McWilliams 1992). However, no mutations affecting eye color have been described for this bug. Normal eye color in adult tarnished plant bugs was described by Haseman (1918) as being purplish-black. Most eye color mutations in insects are recessive involving only one gene (Dustmann 1987). Mutations that result in insects with various types of red eye color have commonly been found (Lindsley and Grell 1968, Bartlett and Lewis 1978, Saul 1982, Vevers 1982, McCombs and Saul 1989, Yamada and Selivon 2001).

In June 1995, a single male adult tarnished plant bug having bright red eyes was collected from annual fleabane, *Erigeron annuus* (L.) Persoon, near Crossett, AR, in Ashley County. This male was used to establish a laboratory colony of red-eye bugs that has been in culture at the Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS, since 1995. The objectives of this study were to evaluate fecundity parameters of the red-eye plant bugs in comparison with normal-eye bugs and crosses of the two strains, and to determine if the red-eye phenotype is caused by a recessive allele at an autosomal locus.

Materials and Methods

Laboratory colonies of the tarnished plant bug have been maintained according to the method described by Snodgrass and McWilliams (1992). The colony of red-eye plant bugs was established by allowing the single adult male collected in June 1995 to mate with virgin females from a laboratory colony. Offspring of these matings were 42 males and 36 females all with normal eye color. They were allowed to interbreed and produced several hundred offspring of which 60 (37 males and 23 females) had red eyes. These red-eye adults were used to establish a colony of plant bugs with red eyes. The red eye color has persisted in the colony for over five years (>40 generations). In comparison with color chips in Ridgway (1912), the phenotype of red-eye insects most closely resembles coral red. The red-eye phenotype is designated in crosses as R, and the normal-type eye color as N. The gene symbol assigned is *r*.

Natality. A first test was conducted to determine if egg production in normal-eye laboratory colony bugs was the same as in red-eye bugs and in matings between red-eye and normal-eye bugs. Adult virgin male and female plant bugs 8–9 d old (sexually mature [Bariola 1969]) were paired and each pair was placed into a 29.5-ml clear plastic cup (Solo Cup Company, Urbana, IL), which contained a 3.5-cm piece of green bean, *Phaseolus vulgaris* L., pod for food and oviposition. The cup was sealed with a cardboard lid with the center cut out and replaced with organdy cloth to

provide ventilation. Virgin plant bugs in this and subsequent tests were obtained by separating the plant bugs by sex when they reached fourth or fifth instar (in these instars the ovipositor of the female is easily seen under low magnification). Recriprocal crosses of red-eye and normal-eye bugs as well as crosses of pure red × red and normal-eye × normal-eye were made using 30 pairs in each of the four crosses. The green bean pieces were changed daily for a 4-wk period and the number of eggs laid was recorded daily for each pair. Eggs were counted by examining each green bean piece under magnification. Eggs were inserted by females into the bean tissue, but the operculum of each egg protruded above the surface of the bean and was easily seen. Rearing of adults, nymphs, and eggs in this and in the following tests was performed at 27°C, 65 ± 10% RH, and a photoperiod of 14:10 (L:D) h. Egg production data were compared by analysis of variance (SAS Institute 1997) using a completely random design. Each cross was a treatment and the experimental unit was the male and female in each cup. Mean cumulative egg production in each of the four crosses was calculated by suming the number of eggs laid in a day and dividing the total by the number of females which were alive at the beginning of the 24-h period. Each day the mean number of eggs was added to the mean number from the previous 24-h period. Mean cumulative egg production was compared on days 5, 10, 15, 20, and 25 of the test.

Egg Viability. Egg viability was determined in a second test. Each of the four crosses described in the first test were again made by placing 20 virgin males and 20 virgin females from each cross into feeding-oviposition cages for egg collection as described by Snodgrass and McWilliams (1992). This rearing method allowed for eggs to be extracted from the tissue paper into which they had been laid. Eggs were held on moist filter paper counted, and observed for development and hatch. Eggs were collected every two d for a 10-d period during which ≈1,000 eggs were obtained for each of the four crosses. Percentage egg hatch and eye color of nymphs was determined for each cross. Percentage egg hatch was calculated by dividing the number of eggs that hatched by the total number of eggs that showed development. Eggs that showed development were used instead of total number of eggs because part of the eggs were damaged during the extraction process, and damaged eggs were difficult to distinguish from infertile eggs.

Genetics. F_1 adults from all four crosses were either interbred to produce F_2 progeny or backcrossed to each of the parental strains (normal-eye and red-eye). In each cross used to produce F_2 progeny, and in the backcrosses, 20 virgin males and 20 virgin females were placed together in 3.79-liter cardboard cartons with organdy cloth tops. Whole green bean pods were used for food and oviposition. The beans were changed every 2–3 d and held in separate containers to record egg hatch. The eye color of the resulting nymphs was determined within 72 h after they hatched by anesthetizing them with CO_2 and examining them under magnification. Sex of the nymphs

was determined when they reached the fourth or fifth instar. The test was conducted for four weeks. If red eyes are controlled by a simply inherited autosomal recessive allele, then segregation ratios for the F_2 and backcross progenies should be 3:1 and 1:1 (normal eye to red eye), respectively. Segregation ratios of progeny obtained in the test crosses were compared with expected ratios using PROC FREQ of SAS (SAS Institute 1997), which computes an asymptotic test for binomial proportions (Z-test). The ratio of male to female offspring with each eye color was also compared with this test.

Field Survey for Red-Eye Mutant. For a measure of how frequently the red-eye phenotype occurred in wild populations, tarnished plant bug adults and nymphs were collected with a sweep net from wild host plants in the vicinity of Crossett, AR, where the single red-eye male was collected in June 1995. These collections were made on 6, 15, and 21 November 1996 and 16 April, 19 May, and 13 June 1997. All adults and nymphs collected were taken to the laboratory, where they were anesthetized with CO_2 and examined under magnification for eye color. Also, in conjunction with a large study on pyrethroid resistance in tarnished plant bugs (Snodgrass and Scott 2000), bugs from 71 sample locations in the Mississippi River Delta of Arkansas, Louisiana, and Mississippi were collected from weeds in the spring and fall from 1995 to 1997. Before being tested for resistance, the eye color of all adults and nymphs collected was determined. Voucher specimens of the red-eye mutation were deposited in the insect collection of the Mississippi Entomological Museum, Mississippi State University, Mississippi State, MS.

Results

Natality. Red-eye females crossed with red-eye or normal-eye males laid as many eggs as did normal-eye females mated with normal-eye or red-eye males (Table 1). No significant differences ($P \geq 0.05$) in mean cumulative egg production were found among the four crosses for any of the five dates. In the red-eye × red-eye and normal-eye × normal-eye crosses, three females in each cross (10.0%) did not lay eggs. This compares to five (16.7%) and six (20.0%) females that did not lay eggs in the normal-eye male × red-eye female and red-eye male × normal-eye female crosses, respectively.

Egg Viability. All offspring from reciprocal crosses (1,234) had normal eye color as did the offspring from the normal-eye × normal-eye cross (614). Offspring from the red-eye × red-eye cross (674) all had red eyes. Percentage egg hatch and standard deviations for eggs from the normal-eye × normal-eye, red-eye × red-eye, red-eye male × normal-eye female, and red-eye female × normal-eye male crosses were 74.1 ± 3.8, 77.2 ± 3.9, 73.8 ± 5.7, and 75.9 ± 4.4, respectively, and were all very similar.

Genetics. Segregation ratios for the F_2 and backcross progenies are given in Table 2. None of the segregation ratios obtained in the crosses were signif-

Table 1. Mean cumulative numbers of eggs (±SE) per female (n) produced in pure or reciprocal crosses of normal-eye (N) and mutant red-eye (R) tarnished plant bugs over a 4-wk period

Cross ♂ × ♀	Day									
	5	$n^{a,b}$	10	n	15	n	20	n	25	n
N × N	31.9 (4.2)	27	62.9 (6.9)	23	96.8 (9.3)	20	120.3 (11.6)	17	130.2 (13.0)	5
R × R	21.6 (4.0)	27	53.6 (6.9)	26	98.6 (9.2)	26	143.8 (11.5)	23	170.5 (13.0)	15
N × R	21.9 (4.3)	25	66.0 (7.3)	25	99.8 (9.8)	19	136.2 (12.1)	13	146.5 (13.5)	5
R × N	20.9 (4.2)	24	72.5 (7.2)	24	111.8 (9.6)	21	123.4 (12.4)	17	135.2 (13.8)	11
F	1.63		2.42		0.73		0.03		0.51	
P > F	0.21		0.12		0.39		0.85		0.48	

[a] Although the test began with 30 females in each cross, three females in the N × N and R × R crosses, and five and six females in the N × R and R × N crosses, respectively, never laid eggs.
[b] The degrees of freedom for each comparison was 1, 99.

icantly different from those expected for control of red eye color by a simply inherited autosomal recessive mutation ($P = 0.49$–1.0). Numbers of male and female progeny produced in the crosses did not differ significantly with either eye color ($P = 0.47$–1.0).

Field Survey. A total of 3,023 adults and nymphs was collected near Crossett, AR, and examined for red eyes. One male with red eyes was found on 21 November 1996, which was 0.0003% of the population sample. A total of 29,715 (19,538 adults and 10,177 nymphs) was collected as part of the pyrethroid-resistance study in 1995–1997. No individuals with red eyes were found.

Discussion

The area around Crossett, AR, is heavily forested, and areas in which wild hosts of tarnished plant bugs were found were mainly narrow areas along roads and in cut-over areas in which trees had been harvested. The presence of forests could limit movement of plant bugs in or out of these areas which could lead to a smaller genepool and increase the chance for the phenotypic expression of a recessive mutation such as red eyes. Even under these conditions, the phenotypic expression of the mutation was found to be rare. In the

Delta, where wild and cultivated host plants are common and movement is little restricted by barriers, no tarnished plant bugs that expressed the mutation were found. It is not known how far tarnished plant bugs move, since migration in this insect species has not been studied. Information on movement of this insect would be very useful in the design of integrated pest management programs for its control in cotton and other crops. Bartlett and Lingren (1984) used pink bollworms. *Pectinophora gossypiells* (Saunders), with an autosomal dominant body color, sooty, to study the movement and population dynamics of feral populations in untreated, pheromone-treated, and insecticide treated cotton fields. Having a visible genetic marker such as red eyes would be useful in conducting similar studies with tarnished plant bugs in cotton or other crops. Availability of a pheromone trap for recapture of plant bugs would greatly facilitate such studies. However, the pheromone produced by the female to attract males (Scales 1968) has not been identified, and pheromone traps are not available.

Test results showed that the mutant red eye color of the tarnished plant bug was controlled by a single autosomal recessive allele. How the mutation produced the red eye color in the tarnished plant bug was not determined. The mutation apparently gave no

Table 2. Segregation for eye color in F_1 (normal-eye × red-eye) backcross progeny from reciprocal crosses of normal-eye (N) and red-eye (R) forms of the tarnished plant bug

Cross ♂ × ♀	Progeny phenotype						Total		P
	Red-eye		P	Normal-eye		P			
	♂	♀		♂	♀		R	N	
	P_1 normal-eye ♂ × red-eye ♀								
R × F_1	46	48	0.84	50	51	0.92	126	133	0.66
N × F_1	—	—		74	81	0.57	0	225	1.00
F_1 × R	23	23	1.00	30	26	0.59	61	64	0.49
F_1 × N	—	—		113	105	0.59	0	384	1.00
F_1 × F_1	24	28	0.58	72	79	0.57	79	245	0.80
	P_1 red-eye ♂ × normal-eye ♀								
R × F_1	51	44	0.47	53	48	0.62	140	140	1.00
N × F_1	—	—		90	106	0.25	0	335	1.00
F_1 × R	40	34	0.49	28	31	0.70	89	95	0.66
F_1 × N	—	—		38	43	0.58	0	141	1.00
F_1 × F_1	26	30	0.59	91	83	0.54	92	256	0.54

N, normal-eye color; R, red-eye phenotype. P, Probability of significance based on comparison of actual ratio to expected ratio (Z-test). Total number with red- or normal-eye color determined after egg hatch. This total does not match totals under ♂s and ♀s with the two eye colors because of nymphal mortality prior to sex determination (fourth–fifth instar).

competitive advantage to mutant individuals, because they were found at very low levels in the population at Crossett, AR, in 1995 and again in 1996 and 1997. No abnormalities in behavior or physiology associated with the red-eye trait were found in the laboratory studies. An eye color mutation can affect vision-dependent behavior in insects such as mating (Chatterjee and Singh 1988). However, the mutant red-eye plant bugs were reared in relatively small containers where the importance of vision in finding food or in mating would be less important than it could be in nature. The higher numbers of females that did not lay eggs found in the crosses between red-eye males and females with normal-eye males and females could represent a low level of mating incompatibility. However, some of these females or males could have simply been injured when they were placed in the rearing cups, or when the green bean pieces were changed.

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